Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method of mixing heterologous genes in expression cassettes located on artificial chromosomes said method comprising the steps of
providing two initial populations of cells that can mate with each other,
said initial populations comprising at least 2 cells in each population, and at least two cells in one ~~each~~ population having different combinations of heterologous genes and/or different combinations of expression cassettes,
each cell comprising at least a first type of artificial chromosome, the at least first type of artificial chromosome comprising both at least two expression cassettes comprising heterologous genes and at least one selectable marker,
the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for,
mating the cells with each other, and

selecting mated cells that carry at least a subset of the selectable markers present on the artificial chromosomes in the two initial populations.

2. (Original) The method of claim 1, further comprising causing the selected mated cells to undergo meiosis.

3. (Cancelled)

4. (Original) The method according to claim 1, wherein the subset of markers selected for comprises at least one marker from and artificial chromosome in each of the initial populations to ensure selection of mated cells.

5. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the selection for a subset of the selectable markers includes selecting at least 70 % of all diploid types present in the mated population.

6. (Cancelled)

7. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, further comprising screening mated cells for one or more parameters related to a desired

functionality(ies) and selecting cells having a predefined selection criterion(a) to undergo meiosis and mating.

8. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, further comprising screening cells that have undergone meiosis for at least one parameter related to a desired functionality(ies) and selecting cells having a predefined selection criterion(a) to undergo mating and meiosis.

9. (Currently amended) The method according to claim~~s 7 or~~ 8, wherein the selection threshold(s) associated with the desired functionalty(ies) is increased for each round of mating and meiosis.

10. (Cancelled)

11. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 2, further comprising repeating the steps of claim~~s 1 and~~ 2 at least twice.

12. - 14. (Cancelled)

15. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, further comprising separating cells of the two mating types from each other after meiosis.

16. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, further comprising mixing spores from different populations prior to mating.

17. (Cancelled)

18. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 2, further comprising adding a further population of cells with types of artificial chromosomes comprising at least two expression cassettes with heterologous genes, the cells being capable of mating with the cells that have undergone mating and meiosis, the further population comprising at least 2 cells with combinations of expression cassettes different from the combinations in the cells of the initial population, the artificial chromosomes of said further population carrying at least one selectable marker.

19. - 22. (Cancelled)

23. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein at least one of the two initial populations of cells that can mate with each other further carry at least a second type of artificial chromosome with expression cassettes comprising heterologous genes, the first and second types of artificial chromosome carrying at least one selectable marker so that said first and second type of artificial chromosome can be individually selected for.

24. - 25. (Cancelled)

26. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the two initial populations of cells that can mate with each other carry from 1 to 10 types of artificial chromosomes, each type of artificial chromosome of each population carrying at least one selectable marker so that each of the types of artificial chromosomes from each of the two populations can be individually selected for.

27. (Cancelled)

28. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein each cell carries 2 artificial chromosomes per cell that can mate.

29. (Cancelled)

30. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein each artificial chromosome carries at least two selectable markers, the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for.

31.- 35. (Cancelled)

36. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the two initial populations are of different mating types.

37. - 38. (Cancelled)

39. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein type of artificial chromosomes with the same marker or combination of markers differ with

respect to combinations of expression cassettes comprising heterologous genes.

40. - 42. (Cancelled)

43. (Currently amended) The method according to claim ~~42~~ 1, wherein the species of cells is fungal cells ~~are~~ selected from a spore forming species.

44. (Currently amended) The method according to claim 1 ~~42~~, wherein the species of ~~fungal~~ cells ~~are~~ is yeast cells.

45. - 48. (Cancelled)

49. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the mated cells are diploid or tetraploid or hexaploid.

50.- 51. (Cancelled)

52. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the expression cassettes are located on a nucleotide concatemer comprising in the 5′→3′

direction a cassette of nucleotide sequence of the general formula

$$[rs_2\text{-}SP\text{-}PR\text{-}X\text{-}TR\text{-}SP\text{-}rs_1]_n$$

wherein

rs_1 and rs_2 together denote a functional restriction site,

SP individually denotes a spacer of at least two nucleotide bases,

PR denotes a promoter, capable of functioning in a cell,

X denotes an expressible nucleotide sequence,

TR denotes a terminator, and

SP individually denotes a spacer of at least two nucleotide bases, and

$n \geq 2$, and

wherein at least a first cassette is different from a second cassette.

53. (Cancelled)

54. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, comprising nucleotide sequences from at least two expression states.

55. (Cancelled)

56. (Original) The method according to claim 52, wherein the rs_1-rs_2 restriction site of essentially all cassettes are recognised by the same restriction enzyme,.

57. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein substantially all expression cassettes on one artificial chromosome are different.

58. (Original) The method according to claim 52, wherein at least one expression cassette comprises an intron between the promoter and the expressible nucleotide sequence.

59. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, wherein the different combinations of expression cassettes comprises different promoters, and/or different expressible nucleotide sequences, and/or different spacers and/or different terminators and/or different introns.

60. (Original) The method according to claim 52, wherein n is at least 10,.

61. (Cancelled)

62. (Original) The method according to claim 54, wherein the different expression states represent at least two different tissues, such as at least two organs, such as at least two species, such as at least two genera.

63. - 65. (Cancelled)

66. (Original) A method of mixing heterologous genes in expression cassettes located on artificial chromosomes said method comprising the steps of
providing two initial populations of protoplasts or cells that can be fused,
said initial populations comprising at least 2 cells in each population, and at least two cells in each population having different combinations of heterologous genes and/or different combinations of expression cassettes,

each cell comprising at least a first type of artificial chromosome, said at least first type of artificial chromosome comprising both at least two expression cassettes comprising heterologous genes and at least one selectable marker,

the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for,

performing protoplast fusion and regeneration of cell walls or performing fusion of cells, and

selecting fused cells that carry at least a subset of the selectable markers present on the artificial chromosomes in the two initial populations.

67. (Currently Amended) The method according to claim 66, further comprising repeating the steps ~~of claim 66~~ thereof.

68. (Currently Amended) The method according to claim 66, wherein the species of cells are selected from fungi, algae, plants, prokaryotes, animal cells or human cells.

69.- 71. (Cancelled)

72. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 66 ~~to 71~~, further comprising screening cells that result from protoplast fusion for a desired functionalty(ies) and selecting cells having the desired functionalty(ies) above a defined threshold, isolating protoplasts from these cells and performing protoplast fusion and cell regeneration on the selected cells.

73. - 75. (Cancelled)

76. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 66~~ to 75~~, wherein at least one of the two initial populations of protoplasts that can fuse with each other further carries at least a second type of artificial chromosome with expression cassettes comprising heterologous genes, the first and second type of artificial chromosome from each population carrying at least one selectable marker so that said first and second type of artificial chromosome can be individually selected for.

77. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 66~~ to 76~~, wherein selection of a subset of the selectable markers includes selection for at least 70 % of all fused cell types present in the fused population.

78. - 79. (Cancelled)

80. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 1, further comprising subjecting the populations of cells to physical isolation of artificial chromosomes from the populations for every 2-3 rounds of meiosis and selection, and transferring the isolated artificial chromosomes into new host cells.

81. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 66~~ to 80~~, wherein the two initial populations of cells carry from 1 to 10 types of artificial chromosomes, each type of artificial chromosome of each population carrying at least one selectable marker so that each of the types of artificial chromosomes from each of the two populations can be individually selected for.

82. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 66~~ to 81~~, further comprising adding a further population of cells with artificial chromosomes comprising at least two expression cassettes with

heterologous genes, the cells being capable of fusing with the cells that have undergone fusion, the further population comprising at least 2 cells with combinations of expression cassettes different from the combinations in the cells of the initial population, the artificial chromosomes of said further population carrying at least one selectable marker.

83. (Original) The method according to claim 82, wherein the further population of cells with artificial chromosomes capable of fusing with the cells that have undergone mating and meiosis carry from 1 to 10 types of artificial chromosomes, each type of artificial chromosome of said further population carrying at least one selectable marker so that each of the types of artificial chromosomes can be individually selected for.

84. - 86. (Cancelled)

87. (Original) A method of mixing heterologous genes in expression cassettes located on artificial chromosomes, said method comprising the steps of

a) obtaining at least one population of cells, the cells of said at least one population comprising

a concatemer of expression cassettes of the following formula:

$[rs_2\text{-}SP\text{-}PR\text{-}X\text{-}TR\text{-}SP\text{-}rs_1]_n$

wherein

rs_1 and rs_2 together denote a restriction site,

SP individually denotes a spacer,

PR denotes a promoter, capable of functioning in the cells,

X denotes an expressible nucleotide sequence,

TR denotes a terminator, and

$n \geq 2$,

the cells differing from each other with respect to combinations of expressible nucleotide sequences and/or promoters,

b) isolating at least some of the cassettes of the selected cells by cutting the concatemers with a restriction enzyme cleaving rs_1rs_2,

c) amplifying at least some of the isolated cassettes,

d) assembling the expression cassettes of step c) into artificial chromosomes, and

e) optionally transferring the artificial chromosomes into host cells.

88. (Original) The method according to claim 87, wherein amplification of isolated cassettes comprises PCR with primers for tagging rs_1 and rs_2.

89. (Original) The method according to claim 87, wherein amplification of isolated cassettes comprises inserting isolated cassettes into a vector having a cloning site compatible with rs_1rs_2 and multiplying this vector in a suitable host.

90. (Original) The method according to claim 87, further comprising adding further cassettes for the assembly step.

91. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 87~~ to 90~~, further comprising screening cells with assembled artificial chromosomes for a desired functionalty(ies) and selecting cells having the desired functionalty(ies).

92. (Original) The method according to claim 91, further comprising subjecting the selected cells to further

isolation and amplification of cassettes and assembly of artificial chromosomes.

93. (Original) A method for mixing heterologous genes in expression cassettes located on artificial chromosomes, said method comprising the steps of

providing two initial populations of cells,
said initial populations comprising at least 2 cells in each population, and at least two cells in each population having different combinations of heterologous genes and/or different combinations of expression cassettes,
each cell comprising at least a first type of artificial chromosome, the at least first type of artificial chromosome comprising both at least two expression cassettes comprising heterologous genes and at least one selectable marker,
the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for,
mating the cells with each other,
amplifying the artificial chromosomes in the host cells,
isolating the artificial chromosomes,
mixing the isolated artificial chromosomes,

transferring subsets of said isolated and mixed artificial chromosomes into host cells, and

selecting cells that carry at least a subset of the selectable markers present on the artificial chromosomes in the two initial populations.

94. (Original) The method according to claim 93, further comprising repeating the mixing process at least once.

95. (Original) The method according to claim 93, wherein the host cells into which the subsets of mixed type of artificial chromosomes are transferred already contain artificial chromosomes with expression cassettes with heterologous genes.

96. (Original) A method of mixing expressible nucleotide sequences, said method comprising the steps of

a) obtaining at least one population of cells, the cells of said at least one population comprising at least one expression cassettes of the following formula:

$[rs_2\text{-SP-PR-rs1'-X-rs2'-TR-SP-}rs_1]_n$

wherein

rs_1 and rs_2 together denote a restriction site,

rs1′ and rs2′ together denote a different restriction site,

SP individually denotes an optional spacer,

PR denotes a promoter, capable of functioning in the cells,

X denotes an expressible nucleotide sequence,

TR denotes a terminator, and

$n \geq 2$,

b) isolating at least some of the expressible nucleotide sequences of the selected cells by cutting the cassettes with a restriction enzyme cleaving rs1′rs2′, or by amplifying the sequences with primer pairs templating sequences in rs1′ and rs2′,

c) re-inserting the expressible nucleotide sequences into other similar backbone,

d) re-mixing the expression cassettes, and

e) transferring the re-expression cassettes into host cells.

97. (Original) The method according to claim 96, wherein the isolated expressible nucleotide sequences are inserted into primary vectors comprising a nucleotide sequence cassette of the general formula in 5′→3′ direction:

[RS1-RS2-SP-PR-CS-TR-SP-RS2′-RS1′]

wherein

RS1 and RS1' denote restriction sites,

RS2 and RS2' denotes restriction sites different from RS1 and RS1',

SP individually denotes a spacer sequence of at least two nucleotides,

PR denotes a promoter,

CS denotes a cloning site,

TR denotes a terminator.

98. (Currently amended) The method according to ~~any of the preceding~~ claim~~s~~ 96~~ or 97~~, further comprising mixing artificial chromosomes by the steps of

providing two initial populations of cells that can mate with each other,

said initial populations comprising at least 2 cells in each population, and at least two cells in each population having different combinations of expression cassettes as defined in said claim~~ 96~~,

each cell comprising at least a first type of artificial chromosome, the at least first type of artificial chromosome comprising both at least two expression cassettes comprising heterologous genes and at least one selectable marker,

the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for,
mating the cells with each other, and
selecting mated cells that carry at least a subset of the selectable markers present on the artificial chromosomes in the two initial populations.

99. (Original) A method of mixing heterologous genes in expression cassettes located on plasmids said method comprising the steps of
providing two initial populations of cells that can mate with each other,
said initial populations comprising at least 2 cells in each population, and at least two cells in each population having different combinations of heterologous genes and/or different combinations of expression cassettes,
each cell comprising at least a first plasmid, the at least first plasmid comprising both at least two expression cassettes comprising heterologous genes and at least one selectable marker,
the selectable markers being allocated to plasmids so that each type of plasmid from each population can be individually selected for,

mating the cells with each other, and

selecting mated cells that carry at least a subset of the selectable markers present on the plasmids in the two initial populations.

100. (Original) The method according to claim 99, wherein the expression cassettes are located on a nucleotide concatemer comprising in the 5′→3′ direction a cassette of nucleotide sequence of the general formula

$$[rs_2\text{-}SP\text{-}PR\text{-}X\text{-}TR\text{-}SP\text{-}rs_1]_n$$

wherein

rs_1 and rs_2 together denote a functional restriction site,

SP individually denotes a spacer of at least two nucleotide bases,

PR denotes a promoter, capable of functioning in a cell,

X denotes an expressible nucleotide sequence,

TR denotes a terminator, and

SP individually denotes a spacer of at least two nucleotide bases, and

$n \geq 2$, and

wherein at least a first cassette is different from a second cassette.

101. (Cancelled)

102. (New) The method according to claim 1, wherein at least two cells in each population has different combinations of heterologous genes and/or different combinations of expression cassettes.